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CD7 Expression on CD34+ Cells from Chronic Myeloid Leukaemia in Chronic Phase

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Thirty-seven patients with chronic phase chronic myeloid leukaemia and fourteen healthy controls have been evaluated for lineage differentiation with immunological markers on purified bone marrow CD34 positive cells by multiparameter flow cytometry. The myeloid-associated antigen CD33 and the stem cell factor receptor (CD117, c-kit) was expressed by 82.3% and 73.5% on CP-CML patients and by 57% and 57.5% on healthy donors, respectively ($P < 0.005$). CD34+/CD19+ or CD34+/CD10+ B-lymphoid cell population represented 9.1% and 10.7% of the CD34+ cells in CML whereas in normal controls this subpopulation was expressed by 27.9% and 30.4% of the CD34+ cells, respectively ($P < 0.005$). The T-lineage associated markers (CD7 and CD2) were detected on a minor population of CD34+ BM cells of healthy controls (mean, 3.6% and 4.6%, respectively). The CD2 positive cells represented 1.5% of the CD34+ cells in CML patients. CP-CML patients co-expressed the CD7 antigen on a mean of 32.6% of the CD34+ BM cells. Moreover, 93% of this CD34/CD7 double positive subpopulation co-expressed CD33 antigen in CML patients. Co-expression of CD7 on CD34+ cells was induced to decrease significantly after short-term *in vitro* culture with the differentiation-inducing agent phorbol ester (PMA) and with a combination of cytokines (stem-cell factor, interleukin-3 and granulocyte colony-stimulating factor). In conclusion, a high co-expression of CD7 antigen is demonstrated on CD34+ cells of chronic phase-chronic myeloid leukaemia patients. The loss of CD7 marker following incubation with PMA and cytokines suggests that this antigen is expressed transiently in early myeloid leukaemic CML haemopoiesis. Am. J. Hematol. 61:178–186, 1999.

Key words: CD34; CD7; chronic phase; chronic myeloid; leukemia

INTRODUCTION

Chronic myeloid leukaemia (CML) is a clonal disorder in which the malignant transformation occurs at the level of a pluripotent haemopoietic progenitor cell [1] with involvement of all haemopoietic lineage [1–3]. This condition has an initial chronic phase (CP) characterized by an expanded myeloid clonal haematopoiesis with continued differentiation into mature granulocytes [4]. The CP invariably progresses to a blast crisis (BC-CML), usually within 3 to 5 years from diagnosis [4]. During transformation to acute leukaemia (blast crisis), about 20–30% of cases have B lymphoblastic features with expression of B-lymphatic antigens similar to acute lymphoblastic leukaemia and in a very few cases T lymphoblastic profile predominate [5,6]. The non-lymphoid BC cases in CML represent between 50% to 70% of cases of acute blast transformation and are heterogeneous with regards to lineage markers since the blast cells can co-express different lineage-restricted antigens [7–14]. Because the blast transformation represents the maturation arrest of precursor cells at a given stage of differentiation, the immunophenotype characteristic of the blast cells in BC could reflect the antigen profile of progenitors during CP.

The aim of this work was to evaluate the immunophenotype of CD34+ bone marrow cells for lineage differentiation in chronic phase-chronic myeloid leukaemia.

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We have used the multiparameter flow cytometry approach to analyse co-expression of early lineage-associated antigens [15,16] from CP-CML patients and compare it with the results obtained in BM of healthy individuals.

MATERIALS AND METHODS

Patients

Thirty-seven cases of Ph1 chromosome positive CML, 24 males and 13 females with a median age of 42 (range, 37–64) years were studied since November 1994 until April 1998. All patients were in CP when analyzed. The median time between diagnosis and study was 33 months (1–99). Thirteen patients were studied at diagnosis before starting any treatment. Five cases had been treated with hydroxyurea alone. All these patients had 100% of Ph1-positive metaphases en BM. Nineteen patients had been treated with interferon-a2b (Schering-Plough S.A., Madrid, Spain) for a variable period of time (between 3 and 86 months, median 12). The median time between diagnosis and study in CP was of 35 months (3–99) and 47 months (1–63) in CML patients treated with IFN and hydroxyurea, respectively. Nine out of nineteen IFNα-treated patients had achieved some degree of cytogenetic response with a median of 30% (range, 10–100%) of Ph1-negative metaphases. Seven patients developed blast crisis during the study period. The median time between diagnosis and BC was 54 months (0–71) and between study in CP and BC was 9 months (between 8 and 25). Samples were obtained in accordance to the Institutional Human Research Committee.

Fourteen BM samples from healthy donors for allogeneic transplantation (median age, 46; range, 39–52) were used as controls.

PROGENITOR CELL PURIFICATION

Freshly aspirated BM was collected in preservative-free heparin. Mononuclear cells (MNC) were separated by a Ficoll-Hypaque density gradient (specific gravity 1.077) (Pharmacia, Sweden). After washing twice in Ca^{2+}/Mg^{2+}/red phenol free Hanks' balanced salt solution (HBSS) (Gibco, Paisley, Scotland) supplemented with 1% human serum albumin (Grifols S.A., Barcelona, Spain) ACD-A 6%, pH 7.3, (HBSA) the MNC were enriched for CD34+ cells by using the Miltenyi mini-MACS system (Miltenyi Biotec, Bergisch Gladbach, Germany) with some modifications as previously described (17). Briefly, the MNC were diluted to a concentration of 300×10^6 MNC/ml in HBSA and incubated for 15 min at 4°C with a human IgG1 and an anti-CD34 mouse monoclonal antibody (MAb) (QBEND10) (40 μl/1 × 10^6 CD34+ cells) modified with a hapten. Cells were washed once with HBSA and incubated for 20 min at 4°C with an anti-hapten mouse MAb conjugated to colloidal superparamagnetic microbeads. Cells were then filtered through a nylon mesh (30 μm of diameter) to remove clumps and cell aggregates. The high gradient magnetic columns (MS columns, Mini-MACS, Miltenyi Biotec, Germany) were filled with 1 ml of cold HBSA before use. Labelled cells were applied and the unbound cells were washed out with 0.5 ml ×4 of PBA. Positive cells were eluted from the column outside of the magnet with 1 ml of HBSA. All manipulations were performed in a laminar flow hood and on ice. Cell viability was evaluated with 0.4% Trypan Blue in saline and/or propidium iodide (5 μg/ml, Sigma, St. Louis, MO) (PI).

FLOW CYTOMETRY

Flow cytometry was performed on a Epics-XL-MCL cytometer (Coulter Electronic, Inc., Hialeah, FL) equipped with 488 nm excitation argon laser. Purified CD34+ cells were incubated immediately after purification with AB human serum (0.1 ml/l, vol/vol) for 10 min at 4°C. Afterwards, 1–3 × 10^5 purified CD34+ cells were washed with HBSS with 0.2% of sodium-azide (Merck, Germany) and 2% of bovine serum albumin (Sigma) (HBSS-BSA), vortexed and diluted in a final volume of 50 μl as followed by tube decantation. Phycoerythrin (PE), fluorescein isothiocyanate (FITC) or peridinin chlorophyll protein (PerCP) labeled HPCA2 (Beckton-Dikinson, San Jose, CA) were added to cells at 0.5 μg/ml final concentration. MAbs for double or triple staining were simultaneously added with PE, FITC or PerCP-HPCA2, for 30 min at 4°C in the dark. Samples were washed twice in HBSS-BSA. Dead cells were excluded by gating first on forward scatter (FS) *versus* 7-amino-actinomicin-D (7-AAD, Molecular Probes, Eugene, OR) and second on FS and side scatter (SS) to assay the cell viability and the CD34 cell purity. The cells were acquired by setting a gate around the area of low SS and low to high FS. An amorphous gate identifying the CD34+ cells was drawn in a dual-parameter cytogram of side scatter and fluorescence [18]. FS, SS (linear amplification) and fluorescence signals (logarithmic amplification) were determined for each cell (minimum of 10.000 CD34+ events per sample) and stored in list mode data files. The following direct MAb were used: CD2 (T11; IgG1), CD7 (3A1; IgG2b), CD19 (B4; IgG1), CD10 (J5; IgG2a) (Coulter immunology, Hialeah, FL), CD34 (HPCA-2; IgG1), CD33 (LeuM9; IgG1), HLA-DR (L243, IgG2a) (Beckton Dickinson, San Jose, CA); CD38 (T16, IgG1), CD7 (8H8.1; IgG2a), c-KIT (95C3, IgG1) (Immunotech S.A., Marseille, France). Isotypic controls were IgG1-FITC, IgG2b-PE, IgG2b-FITC (Coulter Immunology), IgG1-PE, PerCP-IgG1 (Becton Dickinson). Cells incubated with isotypic control antibodies of irrelevant specificity conjugated to FITC, PE,

PerCP were used as standards to define the non specific binding in all experiments. Gates defining stained cells were set to include less than 1% of cells stained with the isotypic control. In two-color immunofluorescence experiments, anti-CD34 MAb was labeled with FITC and the 2nd MAb with PE. In experiments involving three-color immunofluorescence, the anti-CD34 was labelled with PerCP, anti-CD7 with FITC and anti-CD33 with PE. Compensation parameters were adjusted at the beginning of the analysis and were set by gating the purified CD34+ cells on FS/SS and aligning mean channels of the single-stained positive population for each color with unstained purified CD34+ cells. Data analysis was performed with the Epics-XL-MCL v. 1.5 workstation software.

IN VITRO CULTURE

Purified BM CD34+ cells were suspended in IMDM medium with 20% fetal calf serum, penicillin and streptomycin. The cells were cultured in 96-well round-bottomed plates (1–2 × 10^5 cells per well) at 37°C with 5% CO_2 in the presence of mitogenic doses of phorbol myristate acetate (PMA 10 ng/ml, Sigma), a combination of cytokines including stem-cell factor (SCF 50 ng/ml; Amgen, Thousand Oaks, CA), interleukina-3 (IL-3 50 ng/ml, Sandoz Basel, Switzerland) and granulocyte colony-stimulating factor (G-CSF 50 ng/ml, Amgen) or without any stimulant. Cells were analyzed for CD7/CD33 expression at 12 hr, 24 hr, 4 days, or 6 days. After washing and blocking with AB human serum, the cells were stained with FITC-CD7, PE-CD33 mAb and 7-AAD or with isotypic control and analysed by flow cytometry.

STATISTICAL ANALYSIS

Results are expressed as arithmetic mean ± standard deviation (SD) Significance levels were determined by two-sided Student's t-test; *P* value lower than 0.05 was considered statistically significant.

RESULTS

CD34+ Cell Purification

Results of the immunomagnetic selection of CD34+ cells were as follows: CD34+ cells represented a 2.5 ± 2.1% and 2.3 ± 0.7% of light density BM cells in CML and controls, respectively. The purity of the CD34+ cells as measured over the whole selected population was 89 ± 7% and 93.8 ± 2.5% in BM-CML and BM of controls, respectively. Cell viability as evaluated by trypan blue exclusion or PI was superior to 90%.

The results obtained by two- and three-color immunofluorescence analysis on purified CD34+ cells were as shown in Table I.

HLA-DR, CD38, and Stem-Cell Factor Receptor (CD117, c-KIT)

Most of the CD34+ BM cells co-expressed the HLA-DR (98.2 ± 1.6%) and CD38 antigens (94 ± 3.6%) in healthy adults and CML patients (HLA-DR 98.6 ± 1.2% and CD38 95.8 ± 4.3%), without differences between them. The stem-cell factor receptor (c-KIT) was expressed by 73.5% of the CD34+ cells from CML, whereas this marker was co-expressed in a mean of 57.5% of the CD34+ cells from controls ($P < 0.005$).

Coexpression of Myeloid CD33 Antigen

Flow cytometry analysis indicated that the myeloid CD33 antigen was expressed on 57 ± 12% of the CD34+ cells in BM from normal controls *versus* 82.3 ± 18% in BM from CP-CML patients ($P < 0.005$).

Coexpression of B-Cell Markers (CD19 and CD10)

In BM of healthy individuals the co-expression of B-associated cell antigens CD19 and CD10 on CD34+ cells was 27.9 ± 10% and 30.4 ± 10%, respectively. This double positive subpopulation CD34/CD19 or CD34/CD10 was not always detectable on CP-CML patients and represented a mean of 9.1% and 10.7% of the CD34+ cells, respectively.

Coexpression of the T-Cell Antigen (CD7 and CD2)

32.6 ± 19% of CD34-positive cells from CP-CML patients co-expressed CD7 T-lymphoid antigen, whereas CD2 was expressed on 1.5 ± 2.2%. Small but clear-cut CD34+CD7+ and CD34+CD2+ subpopulations could be distinguished in bone marrow of controls and they represented between 3.6 ± 1.4% and 4.6 ± 2.4% of the CD34+ cells, respectively. In some experiments, two different anti-CD7 clones 3A1 and 8H8.1 were used and showed similar reactivity on CD34+ cells (data not shown). To investigate whether CD7 expression on CD34+ cells is affected by the isolation procedure, we analyzed their expression before and after the immunomagnetic separation in nine samples from BM-CML patients and in one from healthy adult. The results showed similar proportion of CD7+ expression on CD34+ cells before and after the CD34 cell purification (39.8 ± 13% *vs.* 40 ± 11% in BM-CML, respectively, P = not significant, paired samples Student's t-test, and 6% and 5.4% in BM of healthy adult, respectively).

Coexpression of CD33 on CD34+/CD7+ Cells

We next wanted to investigate the CD34+/CD7+ subpopulation for the coexpression of myeloid lineage asso-

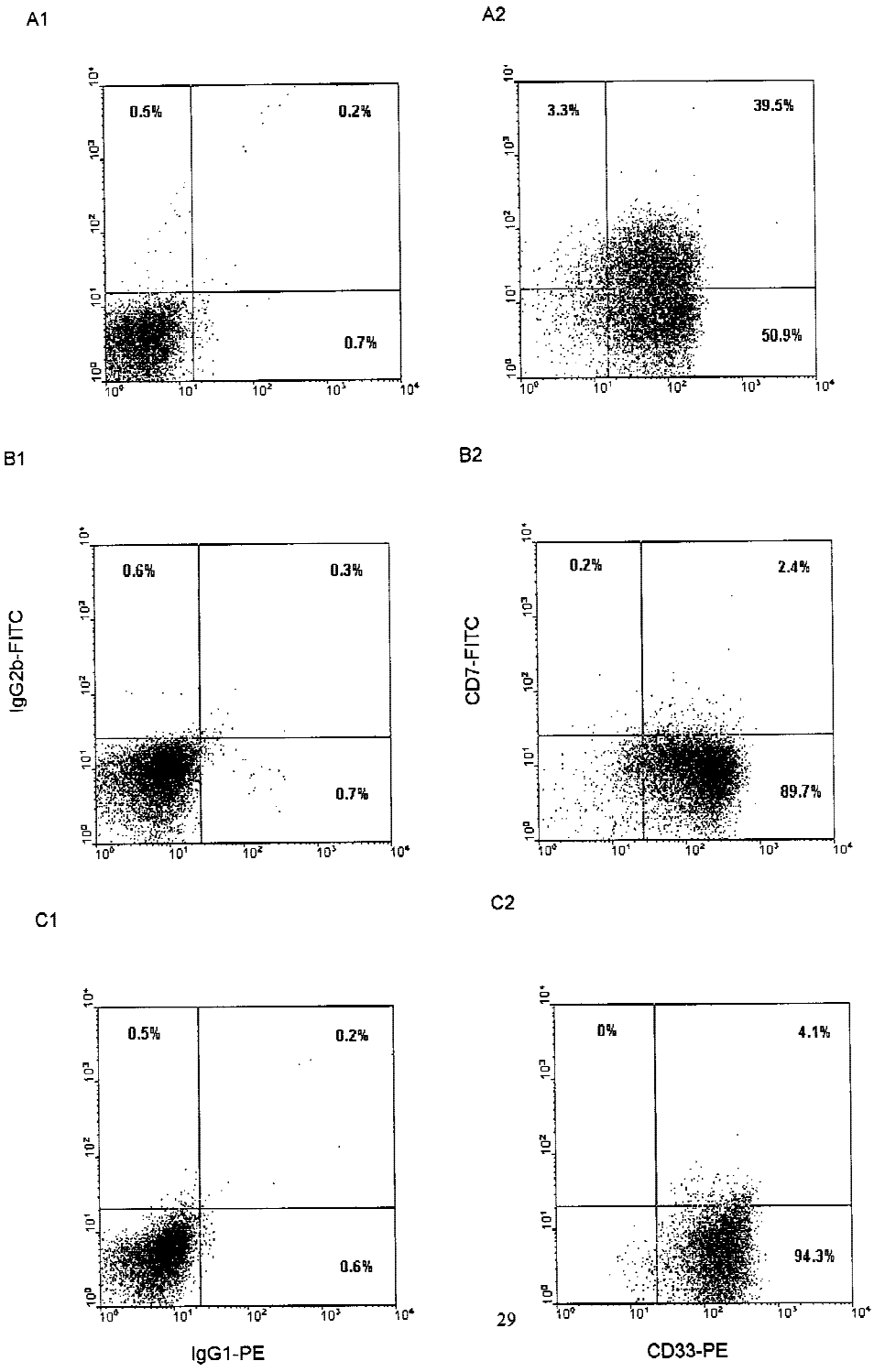

Fig. 1. Dot plots showing CD7 and CD33 expression on purified CD34+ BM cells after in vitro culture in a CP-CML patient. Isotype controls are shown for every condition (A1, B1, C1). Cursors were set to include <1% of the events in the regions of positive events. (A2) Cells cultured in the medium only after 24 hr; (B2) cultured in the presence of PMA after 24 hr; (C2) cultured in the presence cytokines (stem cell factor, IL-3 and G-CSF) after 6 days.

ciated antigens CD33. Using three-color immunofluorescence analysis, CD34+/CD7+ cells coexpressed CD33 marker on 93 ± 6% of the CD34+ cells in CML patients (n = 7; 3 patients at diagnosis, 2 treated with interferon and 2 with hydroxyurea) and 73 ± 15% in BM of healthy individuals (n = 7) ($P < 0.05$).

CD7 Expression after In Vitro Culture

In vitro culture of purified CD34+ BM cells from chronic phase CML was performed in eight independent samples (three at diagnosis, three treated with IFN, and two with hydroxyurea). The CD7/CD33 coexpression on the cells before and after culture is shown in Table II.

TABLE I. Expression of HLA-DR, CD38, c-KIT, and T (CD7 and CD2), B (CD19, CD10) and Myeloid (CD33) Cells Antigens and Co-expression of CD7 and CD33 on Highly Selected CD34+ Cells from Bone Marrow of Chronic Myeloid Leukaemia (BM-CML) and Healthy Adults (BM-Controls)

Mean relative no. of CD34+ cells			
Marker	BM-CML	BM-controls	*p*
HLA-DR+	98.6 ± 1.2	98.2 ± 1.6	NS
CD38+	95.8 ± 4.3	94 ± 3.6	NS
c-KIT+	73.5 ± 16	57.5 ± 13	<0.005
CD33+	82.3 ± 18	57 ± 12	<0.005
CD19+	9.1 ± 14.5	27.9 ± 10	<0.005
CD10+	10.7 ± 15	30.4 ± 10	<0.005
CD7+	32.6 ± 19	3.6 ± 1.4	<0.005
CD2+	1.5 ± 2.2	4.6 ± 2.4	NS
CD7+/CD33+[a]	93 ± 6	73 ± 15	<0.05

Results are expressed as the mean ± SD. BM-CML (bone marrow from chronic myeloid leukemia in chronic phase). All samples were highly purified for CD34+ as described in Materials and Methods.
[a]Percentage of CD34+/CD7+ cells that co-express CD33. Co-expression of CD7/CD33 on CD34 cells was performed by three-color immunofluorescence (PerCP-CD34, FITC-CD7 and PE-CD33) in BM-CML (n = 7) and healthy donors (n = 7).

TABLE II. CD7/CD33 Double Positive Cells on Purified Bone Marrow CD34+ Cells from CP-CML Patients Before and After *In Vitro* Culture

		Percentage of cells with CD7/CD33 expression					
		PMA		Cytokines cocktail		Without stimulant	
UPN	Before culture	12 h-4 d[1]	6 d	12 h-4 d	6 d	12 h-4 d	6 d
56	56	2.5[(c)]	0.3	ND	ND	42	ND
59	21.5	0.9[(b)]	ND	17.4	ND	19.8	ND
69	33	3.8[(c)]	1.2	ND	ND	16	14.7
74	55.1	1[(c)]	0.9	ND	20.3	14	17
81	16.3	ND	2.6	ND	10	ND	15.4
92	23.3	2.7[(a)]	ND	15.7	7.2	15	21.2
101	57.3	2.4[(b)]	ND	ND	4.1	39.5	33.8
102	19.1	2.3[(b)]	ND	ND	1.8	8	0.8
Mean	35.2	2.2	1.3	16.6	8.7	22.0	17.6
SD	18.0	1.0	1.0	1.2	7.2	13.3	11.9

CD34+ BM cells separate by mini-MACS system. UPN 69, 92, and 102 had been studied at diagnosis; 56, 59, and 74 treated with IFN and 81 and 101 with hydroxyurea alone.
[1]Results pooled from different times: [(a)]12 hr; [(b)]24 hr; [(c)]4 d. Results expressed by mean and standard deviation (SD). ND, not done.

The differentiation-inducing agent phorbol ester (PMA) induced a significant decrease of CD7 expression on purified CD34+ cells in all samples and at all the times measured (from a mean of CD33/CD7 double positive cells of 35.2 ± 18% to 2.2 ± 1%, $P = 0.002$, and 1.3 ± 1%, $P = 0.03$, at 4 and 6 days, respectively). Similar results but to a lesser extent were observed when the purified CD34+ cells were incubated with a cocktail of cytokines (SCF, IL-3 and G-CSF) (CD33/CD7 double positive cells decreased from a mean of 35.2 ± 18% before culture to 16.6 ± 1.2%, $P = 0.1$, and 8.7 ± 7.2%, $P = 0.03$, at 4 and 6 days, respectively). CD7 expression was more stable in cells without stimulant although tended to be lost along the culture.

CD7 Expression on CD34+ Cells in Relation to the Previous Treatment

The mean percentage of CD34+ cells expressing CD7 was higher in treated with IFNα (36.9 ± 13.8%) or hydroxyurea (63.5 ± 8.3%) than in recently diagnosed-untreated CP-CML patients (17 ± 13%) ($P < 0.005$) (Table III). No significant differences were demonstrated between untreated, hydroxyurea, or IFNα treated patients with regard to other antigens studied. There was no correlation between the percentage of CD7 positive cells and the duration of disease ($R^2 = 0.001$, $P = 0.9$, linear regression analysis). Forty-seven percent (9 of 19) of patient treated with IFNα showed some grade of cyto-

TABLE III. Immunophenotype Profile of Purified CD34+ Bone Marrow Cells from CP-CML Patients in Relation to Previous Treatment

	Diagnosis	IFN-treated	Hydroxyurea-treated
n	13	19	5
HLA-DR+	98.1 ± 1.1	98.6 ± 1.6	98.6 ± 1.3
CD38+	98.1 ± 1.2	99.1 ± 1	99 ± 1.2
c-KIT+	82.8 ± 13.9	66.3 ± 5.2	79.6 ± 14
CD33+	81.7 ± 18	80.3 ± 21.6	88.4 ± 13.7
CD19+	7.8 ± 15.2	13.2 ± 16.3	9.3 ± 10
CD10+	12 ± 15.7	17.5 ± 18	12 ± 10
CD7+	17 ± 13[a]	36.9 ± 13.8[a]	63.5 ± 8.3[a]
CD2+	0.9 ± 0.6	2.8 ± 3.5	0.5 ± 0.2

[a]There was a statistical difference in the CD7 expression on CD34+ cells in CML patients without previous treatment versus IFNα ($P < 0.005$) and Hydroxyurea treated patients ($P < 0.005$) and between IFNα versus Hydroxyurea treated ($P < 0.005$) patients. No differences were shown in the other antigens studied.

genetic response. No differences were found in CD7 expression on CD34+ cells between cytogenetic response and unresponsive patients (not shown).

Expression of CD7 in Blast Crisis

Seven patients developed blast crisis during the study, two of them at diagnosis. The immunophenotype profile of the blast cell population were as follows: five were myeloid, one was B-lymphoid and one was hybrid myeloid and B-lymphoid. Four out of five myeloid BC coexpressed the CD7 antigen on the blast cell population. Three cases were studied by double labeling with CD34/CD33 and CD34/CD7 over the CD34+ cell blast population. The mean percentage of CD34+ blast cells that coexpressed the CD33 and CD7 antigens was of 57 ± 34% and 28 ± 6%, respectively. A patient was studied by three-color including CD7 and CD33 in the MoAb panel. In this patient, the percentage of CD34+ blasts that coexpressed CD33 and CD7 was 50%. The immunophenotypic study in CP was available in three of five myeloid BC and in two of them the CD34+ cells coexpressed CD7 antigen on 88% and 50% of the cells. Neither B-lymphoid nor the hybrid blast crisis co-expressed the CD7 antigen on blast cells.

DISCUSSION

In this work, we have studied the coexpression of early lineage-associated antigens on purified BM-CD34+ cells from CML in CP and compared it to the results obtained in BM of healthy individuals (Table I). As expected, most of the progenitors cells in CP patients as well as in normal controls are committed as indicated by coexpression of HLA-DR and CD38 on the majority of the CD34+ cells, without differences between them. Similarly, most of the CP-CML patients coexpressed the myeloid-associated CD33 antigen on the bulk of CD34+ cells, reflecting the preferential myelo-expansion of this disease [4]. Similar data were obtained with regards to expression of c-KIT, a surface antigen mostly co-expressed by non-lymphoid progenitor cells [19]. Interestingly, 20% of the CP-CML patients in this serie showed a B-progenitor subpopulation, representing between 5–40% of the CD34+ cells, which suggests that in some patients a B progenitor cell compartment persists within the relative expanded myeloid cell compartment. In previous reports [20,21], BM of healthy individuals shows a 2:1 proportion of myeloid *versus* B-lymphoid progenitor cell compartments (nearly 60% and 30% of the CD34+ cells coexpress CD33 or CD19, respectively). Regarding BM of controls T cell lineage associated antigens (CD7, CD2) expression shows a small but clearly detectable subpopulation of CD34+/CD7+ or CD2+, representing less than 10% of the purified CD34+ cells. Coexpression of CD2 in CP-CML was not always detectable on CD34+ cells and represented a mean of 1.5%. Interestingly, CD34+ BM-cells from CP-CML patients co-expressed the T-cell marker CD7 (mean of 32.6%). The co-expression of antigens normally believed to be restricted to a single lineage on leukaemic blasts has been interpreted as "lineage infidelity" [22] due to aberrant gene expression or "lineage promiscuity" [23] reflecting the existence of a transient phase of limited promiscuity of gene expression occurring in normal early progenitors. The glycoprotein gp40 (CD7) expressed by T and NK cells, is the earliest T-cell lineage-associated antigen to appear during ontogeny and is present on leukaemic cells of T-cell origin [24–26]. Nevertheless, CD7 is also expressed on myeloid blast from some AML and in acute transformation in CML patients [8,9]. Thus, CD7 could also represent an early marker in myeloid ontogeny. In this respect, CD7 has been reported to be expressed by a subgroup of pluripotent progenitor cells in fetal liver and postnatal thymus [24,27,28] and in a small percentage of myeloid BM progenitors [29,30]. We and others [20,31] have also identified a subpopulation in BM of healthy

donors that co-expressed CD7 on CD34+ cells and that this double positive CD34/CD7 subset co-expressed CD33. Thus the co-expression of CD7 and CD33 on CD34+ cells could represent an early stage of normal haemopoietic differentiation. Since CML haemopoiesis is characterized by an expanded myeloid clonal haemopoiesis [32,33], the relative high percentage of CD7 expression on CD34+ cells could well reflect this known characteristic. In fact, this CD34/CD7+ subpopulation mainly co-express the myeloid CD33 antigen on CML, a marker expressed by mix haemopoietic colonies (CFU-Mix) in addition to committed progenitors of erythoid (BFU-E) and granulocyte-macrophage (CFU-GM) lineage's [34].

There was a significant decrease in the CD7 expression on selected CD34+ cells from CML patients when these cells were forced to differentiate with *in vitro* culture containing the differentiating agent PMA and a cytokine cocktail (Table II). These findings further support the hypothesis that CD7 is expressed early and transiently on myeloid leukaemic CML progenitors and that is lost during its differentiation. Previous works from others groups using this approach [31,35] have demonstrated that CD7 was lost in myeloid progenitors from different normal haemopoietic tissues and in leukaemic cells from CD7+ acute myeloblastic leukaemia. At present, the biological significance of this relative high co-expression of CD7 on progenitor cells from CP-CML is unknown. The function of CD7 on normal haemopoietic progenitors also remains to be determined.

A significant higher expression of CD7+ on CD34+ cells was found in patients treated with hydroxyurea (63.5 ± 8.3%) or IFNα (36.9 ± 13.8%) than in recently diagnosed and untreated (mean: 17 ± 13%) patients with CML (Table III). The reason for this differential expression of CD7 depending on previous treated or untreated patients is unclear. CML patients at diagnosis have a relatively increase of the more differentiated myeloid cell progenitor compartment with respect to the more primitive leukaemic progenitor cells. Thus, in untreated recently diagnosed patients, most of the CD34+ cells that co-express CD33 but not CD7 may reflect preferential expansion of a more differentiated maturational stage within the CD34+ population where CD7 expression has been lost. In fact, an increased ratio of mature to primitive CFU-GM and BFU-E is a common feature of recently diagnosed CML patients [36]. This, cytotoxic and IFNα treatment may increase the relative proportion of this CD34+/CD7+ population by preferentially suppressing the more differentiated subpopulation where the CD7 antigen is not expressed. A greater number of early progenitor cells were found in IFNα treated CML patients, suggesting a preferential inhibition of late progenitors development [37]. Some grade of cytogenetic response was achieved in 9 of 19 CML patients treated with IFNα. No correlation was found between the percentage of Ph1 negative metaphases and the expression of CD7 on CD34+ cells (data not shown). Therefore, it seems unlikely that this CD34/CD7 positive subpopulation could discriminate malignant or non-malignant progenitors. In fact, patients with a 100% of metaphases Ph1+ (treated with hydroxyurea or at diagnosis) presented the higher and the lower co-expression of this marker on CD34+ cells, respectively. Indeed, in one case of CML the CD34 positive cells were sorted according to CD7 expression by FACS-sorter and studied for the presence of BCR-ABL transcript by polymerase chain reaction. The two cell populations (CD34+/CD7+ and CD34+/CD7−) were positive for the bcr-abl rearrangement (data not shown). No significant differences were observed between untreated, hydroxyurea-, and IFNα-treated patients with regards to the expression of the other antigens studied.

On the other hand, seven patients developed blast crisis during the period of this study. Morphological and immunological studies demonstrated the myeloid lineage in five cases and four of them co-expressed the CD7 antigen on myeloblast. Interestingly, two out of three patients in myeloid BC and with available immunophenotype studies in CP, co-expressed the CD7 antigen on the CD34+ leukaemic myeloblast without expression of other T- or B-associated markers, suggesting that the blast transformation involved this progenitor level. A high frequency of CD7 expression has been reported in myeloid blast crisis of CML [8,9]. The data presented in this report suggest that CD7+ myeloid blast from acute transformation in CML might well represent the maturation arrest of haemopoietic progenitors at an early stage of myeloid differentiation when CD7 was transiently expressed.

In conclusion, the results presented in this report show that: 1) most of the progenitors from CP-CML patients are committed to myeloid lineage as indicated by co-expression of CD33, HLA-DR and CD38. 2) Most CP-CML patients express the CD7 marker on nearly half of the CD34+ cells and this subpopulation is probably of myeloid lineage as they mainly co-express the myeloid CD33 antigen. 3) The CD7 expression is transiently co-expressed in CML myelopoiesis since its expression decreases after *in vitro* culture with the differentiation-inducing agent PMA and cytokines. Taken together, this data suggest that CD7+ co-expression in CP-CML progenitor cells may reflect the expansion of myeloid progenitors in this disease and that myeloid blast transformation expressing CD7 antigen may well represent the maturation arrest of haemopoietic progenitors at an early stage of myeloid differentiation where CD7 is transiently expressed. At present, the biological significance of CD7 expression on CD34+ cells from CP-CML patients is not clear. Further investigations on the function of this mol-

ecule on progenitor cells will help to elucidate the role of this protein on normal and CML haemopoiesis.

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